



PATENT COOPERATION TREATY



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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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	(PCT Article 36 and Rule 70	ficationofTransmittalofInternational Preliminary
Applicant's or agent's file reference PH-1796-PCT	FOR FURTHER ACTION Examina	ation Report (Form 1 01/2 2
International application No.	International filing date (day/month/year 28 April 2003 (28.04.03)	26 April 2002 (26.04.02)
International Patent Classification (IPC) or C12N 15/09, 1/19, 9/04, 9/10, 9	national classification and IPC 9/50 // C12R 1:645	
Applicant	KIRIN BEER KABUSHIKI KAI	ISHA
This report is also accompamended and are the basis 70.16 and Section 607 of These annexes consist of These annexes consist of This report contains indications I Basis of the rep II Priority III Non-establishm IV Ack of unity of Reasoned state citations and expendence of the contains of the contains and expenses of the citations are citations.	the Administrative Instructions under the a total of sheets. relating to the following items: nent of opinion with regard to novelty, involution ement under Article 35(2) with regard to novelty applications supporting such statement	rectifications made before this Authority (see Rule PCT).
Date of submission of the demand 28 April 2003 (Ì	ompletion of this report 04 November 2003 (04.11.2003)
Name and mailing address of the IPI	Authorize	ed officer
Facsimile No.	Telephor	est Available Copy



aternational application No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT PCT/JP03/05464

I.	Basis	s of the r	eport
1	. With	regard t	o the elements of the international application:*
	\boxtimes		ernational application as originally filed
	一	the des	scription:
İ		pages	, as originally filed
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ŀ	لــــا	pages	, as originally filed
		pages	, as amended (together with any statement under Article 19
		pages	, filed with the demand
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		pages	, filed with the demand
		pages	, filed with the letter of
	Thes	the land the	o the language, all the elements marked above were available or furnished to this Authority in the language in which hal application was filed, unless otherwise indicated under this item. Its were available or furnished to this Authority in the following language
4.			the drawings, sheets/fig
5.		beyond	port has been established as if (some of) the amendments had not been made, since they have been considered to go the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**
	in thi	is report 10.17).	theets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16)
**	Any r	eplaceme	ent sheet containing such amendments must be referred to under item 1 and annexed to this report.

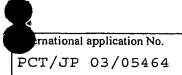


INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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ternational application No.	
PCT/TP03/05/6/	

IV. Lack of unity of invention	
1. In response to the invitation to restrict or pay additional fees the ap	pplicant has:
restricted the claims.	
paid additional fees.	
paid additional fees under protest.	
neither restricted nor paid additional fees.	
2. This Authority found that the requirement of unity of invention not to invite the applicant to restrict or pay additional fees.	on is not complied with and chose, according to Rule 68.1,
3. This Authority considers that the requirement of unity of invention	in accordance with Rules 13.1, 13.2 and 13.3 is
complied with.	
not complied with for the following reasons:	
SEE SUPPLEMENTAL SHEET	
•	
 Consequently, the following parts of the international application we in establishing this report: 	ere the subject of international preliminary examination
all parts.	
the parts relating to claims Nos.	
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT



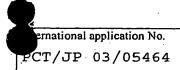
Supplemental Box (To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

The inventions of the present international application can be classified into the following groups:

- (1) claims 1-25 and 94-122: inventions pertaining to methods for producing a methylotroph yeast that is capable of producing a mammalian-type sugar chain;
- (2) claims 26-30: inventions pertaining to an orotidine-5'-phosphate decarboxylase (URA3) gene;
- (3) claims 31-35: inventions pertaining to a phosphoribosyl-amino-imidazole succinocarboxamide synthase (ADE1) gene;
- (4) claims 36-40: inventions pertaining to an imidazole-glycerol-phosphate dehydratase (HIS3) gene;
- (5) claims 41-45: inventions pertaining to a 3-isopropylmalate dehydrogenase (LEU2) gene;
- (6) claims 46-49: inventions pertaining to an α -1,6-mannosyltransferase (OCH1) gene;
- (7) claims 50-53: inventions pertaining to a PEP4 gene;
- (8) claims 54-57: inventions pertaining to a proteinase B (PRB1) gene;
- (9) claims 58-69: inventions pertaining to a YPS1 gene;
- (10) claims 70-73: inventions pertaining to a KTR1 gene;
- (11) claims 74-77: inventions pertaining to an MNN9 gene;
- (12) claims 78-85: inventions pertaining to an alcohol oxidase (AOX) gene; and
- (13) claims 86-93: inventions pertaining to a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

However, the methods for producing a methylotroph yeast that is capable of producing a mammalian-type sugar chain in group (1), and the inventions related to the orotidine-5'-phosphate decarboxylase (URA3) gene in group



Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV.3

(2), the phosphoribosyl-amino-imidazole succinocarboxamide synthase (ADE1) gene in group (3), the imidazole-glycerol-phosphate dehydratase (HIS3) gene in group (4), the 3-isopropylmalate dehydrogenase (LEU2) gene in group (5), the α -1,6-mannosyltransferase (OCH1) gene in group (6), the PEP4 gene in group (7), the proteinase B (PRB1) gene in group (8), the YPS1 gene in group (9), the KTR1 gene in group (10), the MNN9 gene in group (11), the alcohol oxidase (AOX) gene in group (12), and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in group (13) were all well-known prior to the priority date of the present application (refer to "C. Documents Considered to be Relevant" in the international search report); therefore, these features cannot be considered to be special technical features in the light of the prior art.

Consequently, these 13 groups of inventions cannot be considered to be a group of inventions so linked as to form a single general inventive concept.



ternational application No.
PCT/JP 03/05464

Statement			
Novelty (N)	Claims	4-5, 8-9, 15, 17-22, 26-122	YES
	Claims	1-3, 6-7, 10-14, 16, 23-25	NO
Inventive step (IS)	Claims	· · · · · · · · · · · · · · · · · · ·	YES
	Claims	1-122	NO
(2 - 3 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1	Claims	1-122	YES
Industrial applicability (IA)			- NO
	Claims		<u>- NO</u>
Citations and explanations	•		
Document 1: V	WO 02/00856 A2 (Flanders Interuniversity	
	Institute for Bi	otechnology), 03 January	
. 2	2002, claims, ex	amples, & US 2002/188109	A
1	EP 1294910 A2		
Document 2: V	NO 02/00879 A2 (Glycofi Inc.), 03 Januar	У
	2002, claims, ta	ble 3, examples, & US	
	2002/137134 A &	EP 1297172 A2	
Document 3:	Yasuyoshi SAKAI	et al., "The Orotidine-5	′ –
1	Phosphate Decarb	oxylase Gene (URA3) of a	
	Methylotrophic Y	east, Candida boidinii:	
1	Nucleotide Seque	nce and its Expression i	n
j	Escherichia coli	," Journal of Fermentati	on
·	and Bioengineeri	ng, 1992, Vol. 73(4), pa	ges
2	255-260, entire	document, especially fig	. 2
Document 4:	Vina W. YANG et	al., "High-Efficiency	
	Transformation c	of <i>Pichia stipitis</i> Based	on ⁻
	its URA3 Gene an	d a Homologous Autonomou	s
I	Replication Sequ	ence, ARS2," Applied and	
I	Environmental Mi	crobiology, 1994, Vol.	
•	50(12), pages 42	45-4254, entire document	,
•	especially fig.	3	
Document 5:	Yoshiaki NISHIYA	et al., "Primary Struct	ure
		om Candida utilis,"	
Ţ	Rioscience Riote	chnology and Biochemistr	у,

1994, Vol. 58(1), pages 208-210, entire document, especially fig. 3

- Document 6: Inmaculada C. COSANO et al., "Cloning and Sequence Analysis of the Pichia pastoris TRP1, IPP1 and HIS3 Genes," Yeast, 1998, Vol. 14, pages 861-867, entire document, especially fig. 4
- Document 7: WO 98/14600 Al (Centro de Ingenieria y
 Biotecnologia), 09 April 1998, claims, SEQ
 ID NO: 5-6, & JP 2001-501475 A & EP 956356
 Al
- Document 8: Yasuyoshi SAKAI et al., "Directed

 Mutagenesis in an Asporogenous

 Methylotrophic Yeast: Cloning, Sequencing

 and One-step Gene Disruption of the 3
 Isopropylmalate Dehydrogenase Gene (LEU2) of

 Candida boidinii to Derive Doubly

 Auxotrophic Marker Strains," Journal of

 Bacteriology, 1992, Vol. 174(18), pages

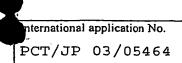
 5988-5993, entire document, especially fig.
- Document 9: Ying-Pei ZHANG et al., "LEU2 Gene Homolog in Kluyveromyces lactis," Yeast, 1992, Vol. 8, pages 801-804, entire document, especially fig. 1
- Document 10: JP 9-3097 A (The Green Cross Corp.), 07

 January 1997, claims, SEQ ID NO: 5, fig. 5,

 (Family: none)
- Document 11: WO 00/14259 A1 (Kirin Brewery Co., Ltd.), 16

 March 2000, claims, SEQ ID NO: 2-3, & JP

 2000-78978 A
- Document 12: WO 92/17595 Al (The Salk Institute
 Biotechnology/Industrial Associates), 15
 October 1992, claims, SEQ ID NO: 1-2, & JP
 6-506117 A & EP 578746 Al & US 5324660 A



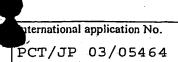
- Document 13: Anahit V. AZARYAN et al., "Purification and Characterization of a Paires Basic Residue-specific Yeast Aspartic Protease Encoded by the YAP3 Gene," The Journal of Biological Chemistry, 1993, Vol. 268(16), pages 11968-11975, entire document
- Document 14: Hiroto KOMANO et al., "Shared Functions in vivo of a Glycosyl-phosphatidylinositol-linked aspartyl protease, Mkc7, and the Proprotein Processing Protease Kex2 in Yeast," Proc. Natl. Acad. Sci. USA, 1995, Vol. 92, pages 10752-10756, entire document, especially fig. 2
- Document 15: Ed T. BUURMAN et al., "Molecular Analysis of CaMntlp, a Mannosyl Transferase Important for Adhesion and Virulence of Candida albicans," Proc. Natl. Acad. Sci. USA, 1998, Vol. 95, pages 7670-7675, entire document, especially fig. 1
- Document 16: EP 314096 A2 (Zymogenetics, Inc.), 03 May 1989, claims, fig. 4, & JP 2-419 A & US 5135854 A & DE 3887082 A
- Document 17: A. M. LEDEBOER et al., "Molecular Cloning and Characterization of a Gene Coding for Methanol Oxidase in Hansenula polymorpha,"

 Proc. Natl. Acad. Sci. USA, 1998, Vol. 95, pages 7670-7675, entire document, especially fig. 6
- Document 18: EP 173378 A2 (Nnilever PLC), 05 March 1986, claims, fig. 11 and 13, & JP 61-92569 A & US 5240838 A & DE 3583194 A
- Document 19: WO 00/78978 Al (Zymogenetics Inc.), 28

 December 2000, claims, SEQ ID NO 1-2, & JP

 2003-503030 A & EP 1192263 Al

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Document 1 discloses a method for producing proteins that have a mammalian-type sugar chain by introducing vectors that express α -1,2-mannosidase into a methylotroph yeast, and specifically discloses features wherein vectors that express α -1,2-mannosidase are introduced into a Pichia-species yeast and the Ochl genes are deactivated. Furthermore, document 1 indicates that AOXI, AOXII, GAP and the like can be selected as the promoter for said vectors, and that ER retention signals are added to the α -1,2-mannosidase genes.

Document 2 discloses a method for producing proteins that have a mammalian-type sugar chain, and specifically discloses the features of producing mutants of Pichia pastorus that do not express OCH1 and of transforming the mutants so that they express mannosidase. Document 2 also indicates that the α -1,2-mannosidase is obtained from microscopic organisms such as $Aspergillus\ saitoi$.

Claims 1-3, 6-7, 10-14, 16 and 23-25 lack novelty in the light of the disclosures of document 1.

Claims 1-3, 10-12 and 23-25 lack novelty in the light of the disclosures of document 2.

In addition, it would be easy for a person skilled in the art to apply these features to $Ogataea\ minuta$, which is one type of methylotroph yeast, to express α -1, 2-mannosidase using a promoter such as AOXI, AOXII or GAP, and to obtain a protein that has a desired N-type sugar chain by deactivating the gene related to the production of the sugar chain and introducing an appropriate heterogeneous gene.

Therefore, it is considered to have been easy for a person skilled in the art to conceive of the inventions that are set forth in claims 1-25 and 94-122 in the light of documents 1-4.

Documents 3-4 disclose the URA3 gene from Candida boidinii and the URA3 gene from Pichia stipitis.

Document 5 discloses the ADE1 gene from Candida utilis.

Documents 6-7 disclose the HIS3 gene from Pichia pastoris and the HIS3 gene from Candida utilis.

Documents 8-9 disclose the LEU2 gene from Candida boidinii and the LEU2 gene from Kluyveromyces lactis.

Document 10 discloses α -1,6-mannosidase from a Pichia-species yeast and the gene that codes said mannosidase, and indicates that this gene is homologous to the OCH1 gene.

Document 11 discloses proteases A and B from Candida boidinii and the genes that code said proteases.

Document 12 discloses protease A from a *Pichia*-species yeast and the gene that codes said protease.

Documents 13-14 disclose the protease YAP3 from yeast and the gene that codes said protease.

Document 15 discloses the KTR1 gene from Saccharomyces cerevisiae.

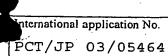
Document 16 discloses the MNN9 gene from Saccharomyces cerevisiae.

Documents 17-18 disclose the MOX gene from Saccharomyces cerevisiae and Hansenula polymorpha.

Document 19 discloses the GAP1 gene from Pichia methanolica, and the promoter and terminator therefor.

On the priority date for the present application, it was common practice in this technical field to synthesize a probe or primer from a portion of a known base sequence that codes a useful natural protein in order to clone the same DNA chain from a different natural source (refer to, for example, Pro. N.A.S., Vol. 72, 1975, pages 3961-3965); therefore, it is not considered to be especially difficult for a person skilled in the art to use probes that are synthesized from portions of the base sequences of each of the genes that are disclosed in





documents 3-19 in order to obtain the URA3 gene, ADE1 gene, HIS3 gene, LEU2 gene, OCH1 gene, PEP4 gene, PRB1 gene, YPS1 gene, KTR1 gene, MNN9 gene, AOX gene, GAPDH gene and the promoters and terminators of the AOX gene and GAPDH gene from Ogataea minuta.

In addition, it is common practice for a person skilled in the art to create recombined vectors that contain said genes, transform *Ogataea minuta* using said recombinant vectors, and produce proteins by cultivating the obtained transformants.

Therefore, it is considered to have been easy for a person skilled in the art to conceive of the inventions that are set forth in claims 26-93 in the light of documents 3-19.